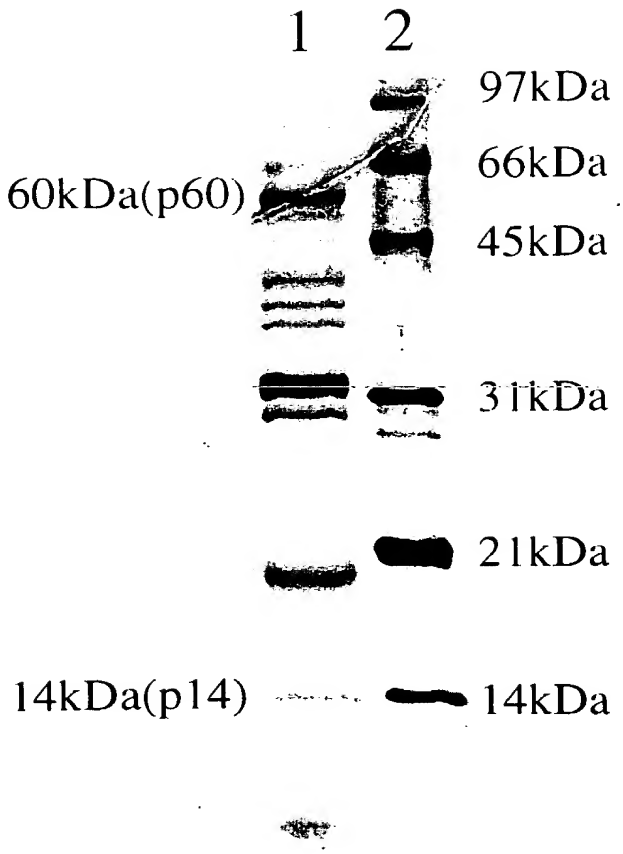
FIG. 1

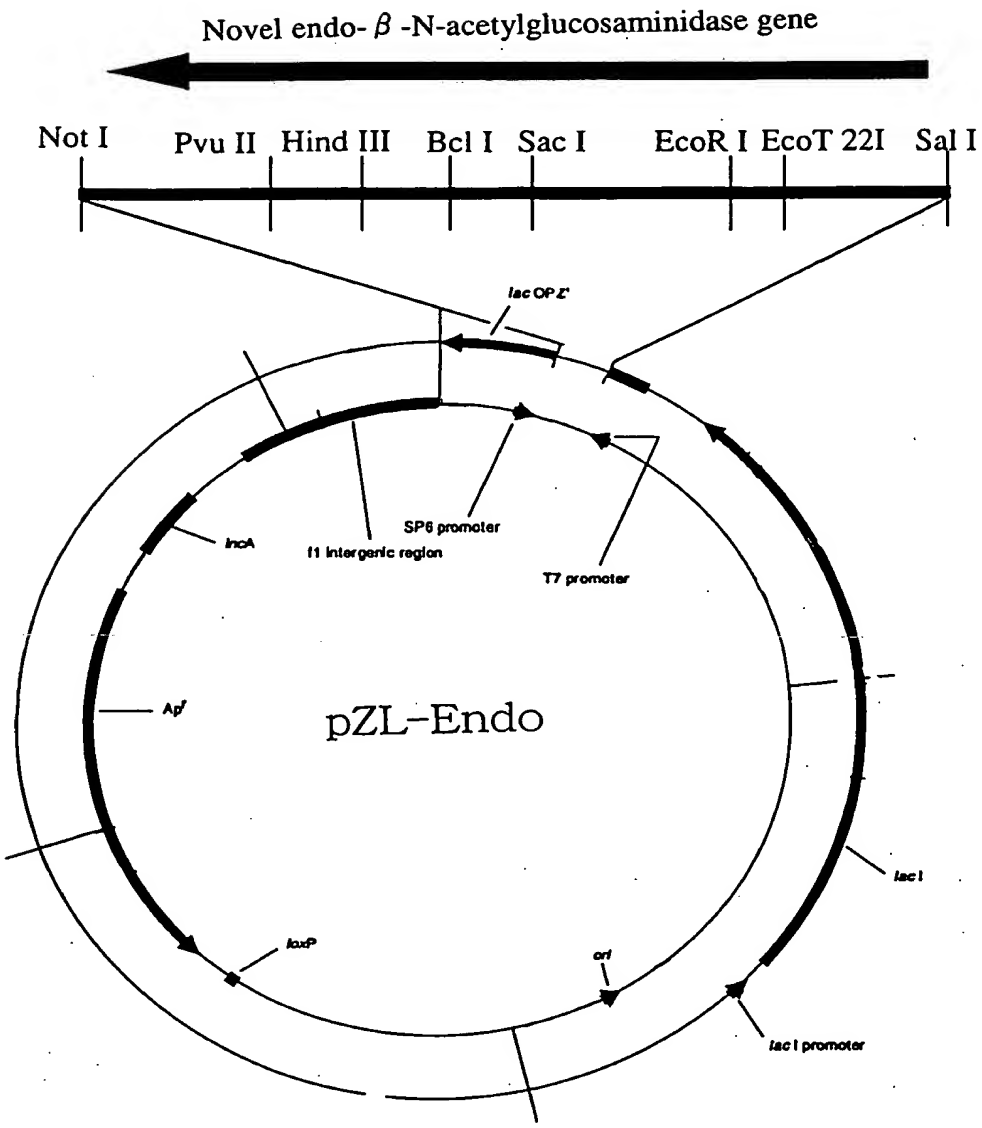


Purification Result for Endo-β -N-acetylglucosaminidase (15-25% gradient SDS-PAGE)

Lane 1: Purified endo-β -N-acetylglucosaminidase from *Mucor hiemalis*

Lane 2: Molecular weight markers

FIG. 2



Restriction enzyme map for pZL-Endo including the full-length novel endo- β -N-acetylglucosaminidase gene.

FIG. 3

```
        10         20         30         40         50         60
GTCGACCCAC GCGTCCGCGG ACGCGTGGGC GGACGCGTGG GCGGACGCGT GGGTTTTATT

        70         80         90        100        110        120
TTACATAAAT ATGCCTTCAC TTCAATTGCA ACCTGATGAC AAACTAGCAC CTGTTTCTTT

       130        140        150        160        170        180
TGCACTTAAG TCTATGAATG AGTTGAGGGA CTGGACGCCA GACGAAAAGA TAAAGTTTAA

       190        200        210        220        230        240
CGTTTCAAGC GTGGCACTAC AGCCTCGTGT GAAAAACGCC CTGAAACCTC AATTATTGTT

       250        260        270        280        290        300
AACTCATGAT ATGGCAGGAG GATATAAAGA AGATAAAAAT ATTCAAGGAA ACAATTATAA

       310        320        330        340        350        360
AGACATTTAT AACATTCAAT ATTGGCATTT AGCTGATACT TTTGTATATT TCTCTCATGA

       370        380        390        400        410        420
GCGAGTTAGC ATTCCTCCAG TCAATTGGAC AAATGCTTGT CATAGAAATG GTGTAAAGTG

       430        440        450        460        470        480
TTTAGGTACT TTTTTAGTAG AAGGAAATAA CCAAATGCAT GAAATGGAAG CCTTGCTTCA

       490        500        510        520        530        540
CGGTCCACCT TTACTTAATA ACACTGACGA CCCTATGAGA TTATGGAGTC CGTATTATGC

       550        560        570        580        590        600
AGACCAATTA GTTGCTATTG CTAAACACTA TGGTTTTGAT GGCTGGTTGT TCAATATTGA

       610        620        630        640        650        660
ATGCGAATTC TTTCCTTTTC CTACAAATCC AAAATTCAAA GCTGAAGAGT TGGCAAAGTT

       670        680        690        700        710        720
TCTACACTAT TTTAAGGAAA AATTGCATAA CGAAATACCT GGATCTCAAC TCATTTGGTA

       730        740        750        760        770        780
CGACAGCATG ACAAATGAAG GAGAAATCCA CTGGCAGAAC CAGCTCACAT GGAAAAATGA

       790        800        810        820        830        840
GTTATTTTTT AAAAACACGG ATGGTATTTT TTTGAATTAT TGGTGGAAAA AAGAATACCC

       850        860        870        880        890        900
TGAAATGGCG CGTAGAGTAG CTGAAGGAAT AGGTAGATCA GGTTTAGAAG TTTATTTTGG

       910        920        930        940        950        960
TACAGATGTA TGGGGAAGGC ATACTTATGG TGGCGGTGGT TTCAAATCAT ATAAGGGTGT

       970        980        990       1000       1010       1020
AAAAACTGCC TACTCTGCAA TGACATCTTC TGCATTATTT GGTATGGCAT GGACATACGA

      1030       1040       1050       1060       1070       1080
GCATTTCGAA AAGTCTGAAT TTGAAAAGAT GGATCGTTTG TTTTGGTGTG GTGGTAAATA

      1090       1100       1110       1120       1130       1140
CTCTGACTAT CCTCCCCCAC CTCCTAAAAA CCCAGATGAC GAAAAAGAAG TAGAAAGCGA

      1150       1160       1170       1180       1190       1200
TGATAGTGAA GATGAGCTCA TGTACGGACA CAAGAAAGGT ATTGCTGACA CGGTAGAATC

      1210       1220       1230       1240       1250       1260
TATTCCTGTA CCAGGAACAG ATTGGTTTGT TACCAATTTT GATAGGGGGT TTGGAAATAG

      1270       1280       1290       1300       1310       1320
GTTTTATTAT AGAGGAAAGA GATTACTTTC TCAGCCTTGG TCCCATTTAT CGCATCAAGC

      1330       1340       1350       1360       1370       1380
TATTCTCCCC AATAAAAGCT ATCGAAATCC AGAGATTTAT CCCACTGATC AAAACATTAA
```

Entire nucleotide sequence of the fragment inserted into the Sal I-Not I sites of pZL-Endo including the full-length novel endo-β -N-acetylglucosaminidase gene.

FIG. 4

```
      1390        1400        1410        1420        1430        1440
AATCACTAGT TCTCTCGATT GCGATCATGG AGCTTTTCTT GGTGGAACCT CGCTTATTAT

      1450        1460        1470        1480        1490        1500
CAAAGGCCAA CGTTTCAATC ATAGAGAATC GCATGATGTT GAAACTGAAA TTAGTATACC

      1510        1520        1530        1540        1550        1560
TCTGTATAAG CTTTCATTAG ATGCTAGTAA AGGATGCTCA TTGCGTTATA TTTATAGAAC

      1570        1580        1590        1600        1610        1620
TTTGTTGATG AAAGATGTAA AGTTGACAGT AGCATGTCAC TTTTCGTTAA AAACAAACGA

      1630        1640        1650        1660        1670        1680
CTCAGTTAAT TTCTTCAAGG TATGGCAGCC AGATGAAAAT TTCTCTTTTG AATATGATGA

      1690        1700        1710        1720        1730        1740
TGGAATGAGA GCCACTGTTA CAACTGAAAA TTCTACCGAA AGCAGATGCT TTTTATTACG

      1750        1760        1770        1780        1790        1800
TACAACAGAA GAAGATACAG GAGAAAATGA TTGGATAACA AAAACTATTA ATGTGCCTGC

      1810        1820        1830        1840        1850        1860
TGTTCCAGAA GGAAGTCAAT TATACATTAC AAGACTTGAA GTGAGCGTAG TATTAGATAC

      1870        1880        1890        1900        1910        1920
AGCTGGTTTA GTAGGTCTTG TTAATCAAGT TATTGCTTGC TTGGGATATA TTAGCATCAT

      1930        1940        1950        1960        1970        1980
ACCAACTATA AATTCTGGAA TAAAAACAGA TTCATCACGC ATTATTCAGG ATCTCTTTTG

      1990        2000        2010        2020        2030        2040
GAAAGATCAG AAATATACCA AAATCGGAAA AGAAAGTTTA GACGACATAG CTCAAGAAGA

      2050        2060        2070        2080        2090        2100
AGTTCATAGA TATTATGGAA CATTGAACTG GGAAAACACA GCAAATGTAG TAAACGCTTG

      2110        2120        2130        2140        2150        2160
GGAGGAAATA GATTACTACA ACGTTTTTTA CAAAGAAAGT GACGACTCTG CAACTCGCAT

      2170        2180        2190        2200        2210        2220
CTTTTTAGGA ACAGCATTCT GTAATCAATT TCGTGTATCT GGTTTAGATA TTATTTTATC

      2230        2240        2250        2260        2270        2280
TAAGCTACCA AAGATAGTTA TTGAAGCTGT TAACAAAGAA GGATACATCT CTTCAAGTGG

      2290        2300        2310        2320        2330        2340
TAGCATAGAT TTGTCATTAA ACTAGGACTT GAAATAAAAT ATTATGATAA AGAAAAAAAA

      2350        2360        2370        2380        2390        2400
AAAAAAAAAA AAAAAAAAAG GGCGGCCGC. .......... .......... ..........
```

Entire nucleotide sequence of the fragment inserted into the Sal I-Not I sites of pZL-Endo including the full-length novel endo-β-N-acetylglucosaminidase gene. (Continued)

FIG. 5

```
                9         18        27        36        45        54
5' ATG CCT TCA CTT CAA TTG CAA CCT GAT GAC AAA CTA GCA CCT GTT TCT TTT GCA
   M   P   S   L   Q   L   Q   P   D   D   K   L   A   P   V   S   F   A

               63        72        81        90        99       108
   CTT AAG TCT ATG AAT GAG TTG AGG GAC TGG ACG CCA GAC GAA AAG ATA AAG TTT
   L   K   S   M   N   E   L   R   D   W   T   P   D   E   K   I   K   F

              117       126       135       144       153       162
   AAC GTT TCA AGC GTG GCA CTA CAG CCT CGT GTG AAA AAC GCC CTG AAA CCT CAA
   N   V   S   S   V   A   L   Q   P   R   V   K   N   A   L   K   P   Q

              171       180       189       198       207       216
   TTA TTG TTA ACT CAT GAT ATG GCA GGA GGA TAT AAA GAA GAT AAA AAT ATT CAA
   L   L   L   T   H   D   M   A   G   G   Y   K   E   D   K   N   I   Q

              225       234       243       252       261       270
   GGA AAC AAT TAT AAA GAC ATT TAT AAC ATT CAA TAT TGG CAT TTA GCT GAT ACT
   G   N   N   Y   K   D   I   Y   N   I   Q   Y   W   H   L   A   D   T

              279       288       297       306       315       324
   TTT GTA TAT TTC TCT CAT GAG CGA GTT AGC ATT CCT CCA GTC AAT TGG ACA AAT
   F   V   Y   F   S   H   E   R   V   S   I   P   P   V   N   W   T   N

              333       342       351       360       369       378
   GCT TGT CAT AGA AAT GGT GTA AAG TGT TTA GGT ACT TTT TTA GTA GAA GGA AAT
   A   C   H   R   N   G   V   K   C   L   G   T   F   L   V   E   G   N

              387       396       405       414       423       432
   AAC CAA ATG CAT GAA ATG GAA GCC TTG CTT CAC GGT CCA CCT TTA CTT AAT AAC
   N   Q   M   H   E   M   E   A   L   L   H   G   P   P   L   L   N   N

              441       450       459       468       477       486
   ACT GAC GAC CCT ATG AGA TTA TGG AGT CCG TAT TAT GCA GAC CAA TTA GTT GCT
   T   D   D   P   M   R   L   W   S   P   Y   Y   A   D   Q   L   V   A

              495       504       513       522       531       540
   ATT GCT AAA CAC TAT GGT TTT GAT GGC TGG TTG TTC AAT ATT GAA TGC GAA TTC
   I   A   K   H   Y   G   F   D   G   W   L   F   N   I   E   C   E   F

              549       558       567       576       585       594
   TTT CCT TTT CCT ACA AAT CCA AAA TTC AAA GCT GAA GAG TTG GCA AAG TTT CTA
   F   P   F   P   T   N   P   K   F   K   A   E   E   L   A   K   F   L

              603       612       621       630       639       648
   CAC TAT TTT AAG GAA AAA TTG CAT AAC GAA ATA CCT GGA TCT CAA CTC ATT TGG
   H   Y   F   K   E   K   L   H   N   E   I   P   G   S   Q   L   I   W

              657       666       675       684       693       702
   TAC GAC AGC ATG ACA AAT GAA GGA GAA ATC CAC TGG CAG AAC CAG CTC ACA TGG
   Y   D   S   M   T   N   E   G   E   I   H   W   Q   N   Q   L   T   W
```

Amino acid sequence deduced from the novel Endo-β-N-acetylglucosaminidase gene, and the nucleotide sequence of the DNA encoding this amino acid sequence.

FIG. 6

```
          711        720        729        738        747        756
AAA AAT GAG TTA TTT TTT AAA AAC ACG GAT GGT ATT TTT TTG AAT TAT TGG TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   N   E   L   F   F   K   N   T   D   G   I   F   L   N   Y   W   W

          765        774        783        792        801        810
AAA AAA GAA TAC CCT GAA ATG GCG CGT AGA GTA GCT GAA GGA ATA GGT AGA TCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   K   E   Y   P   E   M   A   R   R   V   A   E   G   I   G   R   S

          819        828        837        846        855        864
GGT TTA GAA GTT TAT TTT GGT ACA GAT GTA TGG GGA AGG CAT ACT TAT GGT GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   L   E   V   Y   F   G   T   D   V   W   G   R   H   T   Y   G   G

          873        882        891        900        909        918
GGT GGT TTC AAA TCA TAT AAG GGT GTA AAA ACT GCC TAC TCT GCA ATG ACA TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   G   F   K   S   Y   K   G   V   K   T   A   Y   S   A   M   T   S

          927        936        945        954        963        972
TCT GCA TTA TTT GGT ATG GCA TGG ACA TAC GAG CAT TTC GAA AAG TCT GAA TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   A   L   F   G   M   A   W   T   Y   E   H   F   E   K   S   E   F

          981        990        999       1008       1017       1026
GAA AAG ATG GAT CGT TTG TTT TGG TGT GGT GGT AAA TAC TCT GAC TAT CCT CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   K   M   D   R   L   F   W   C   G   G   K   Y   S   D   Y   P   P

         1035       1044       1053       1062       1071       1080
CCA CCT CCT AAA AAC CCA GAT GAC GAA AAA GAA GTA GAA AGC GAT GAT AGT GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   P   P   K   N   P   D   D   E   K   E   V   E   S   D   D   S   E

         1089       1098       1107       1116       1125       1134
GAT GAG CTC ATG TAC GGA CAC AAG AAA GGT ATT GCT GAC ACG GTA GAA TCT ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   E   L   M   Y   G   H   K   K   G   I   A   D   T   V   E   S   I

         1143       1152       1161       1170       1179       1188
CCT GTA CCA GGA ACA GAT TGG TTT GTT ACC AAT TTT GAT AGG GGG TTT GGA AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   V   P   G   T   D   W   F   V   T   N   F   D   R   G   F   G   N

         1197       1206       1215       1224       1233       1242
AGG TTT TAT TAT AGA GGA AAG AGA TTA CTT TCT CAG CCT TGG TCC CAT TTA TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   F   Y   Y   R   G   K   R   L   L   S   Q   P   W   S   H   L   S

         1251       1260       1269       1278       1287       1296
CAT CAA GCT ATT CTC CCC AAT AAA AGC TAT CGA AAT CCA GAG ATT TAT CCC ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   Q   A   I   L   P   N   K   S   Y   R   N   P   E   I   Y   P   T

         1305       1314       1323       1332       1341       1350
GAT CAA AAC ATT AAA ATC ACT AGT TCT CTC GAT TGC GAT CAT GGA GCT TTT CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   Q   N   I   K   I   T   S   S   L   D   C   D   H   G   A   F   L

         1359       1368       1377       1386       1395       1404
GGT GGA ACC TCG CTT ATT ATC AAA GGC CAA CGT TTC AAT CAT AGA GAA TCG CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   G   T   S   L   I   I   K   G   Q   R   F   N   H   R   E   S   H

         1413       1422       1431       1440       1449       1458
GAT GTT GAA ACT GAA ATT AGT ATA CCT CTG TAT AAG CTT TCA TTA GAT GCT AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   V   E   T   E   I   S   I   P   L   Y   K   L   S   L   D   A   S
```

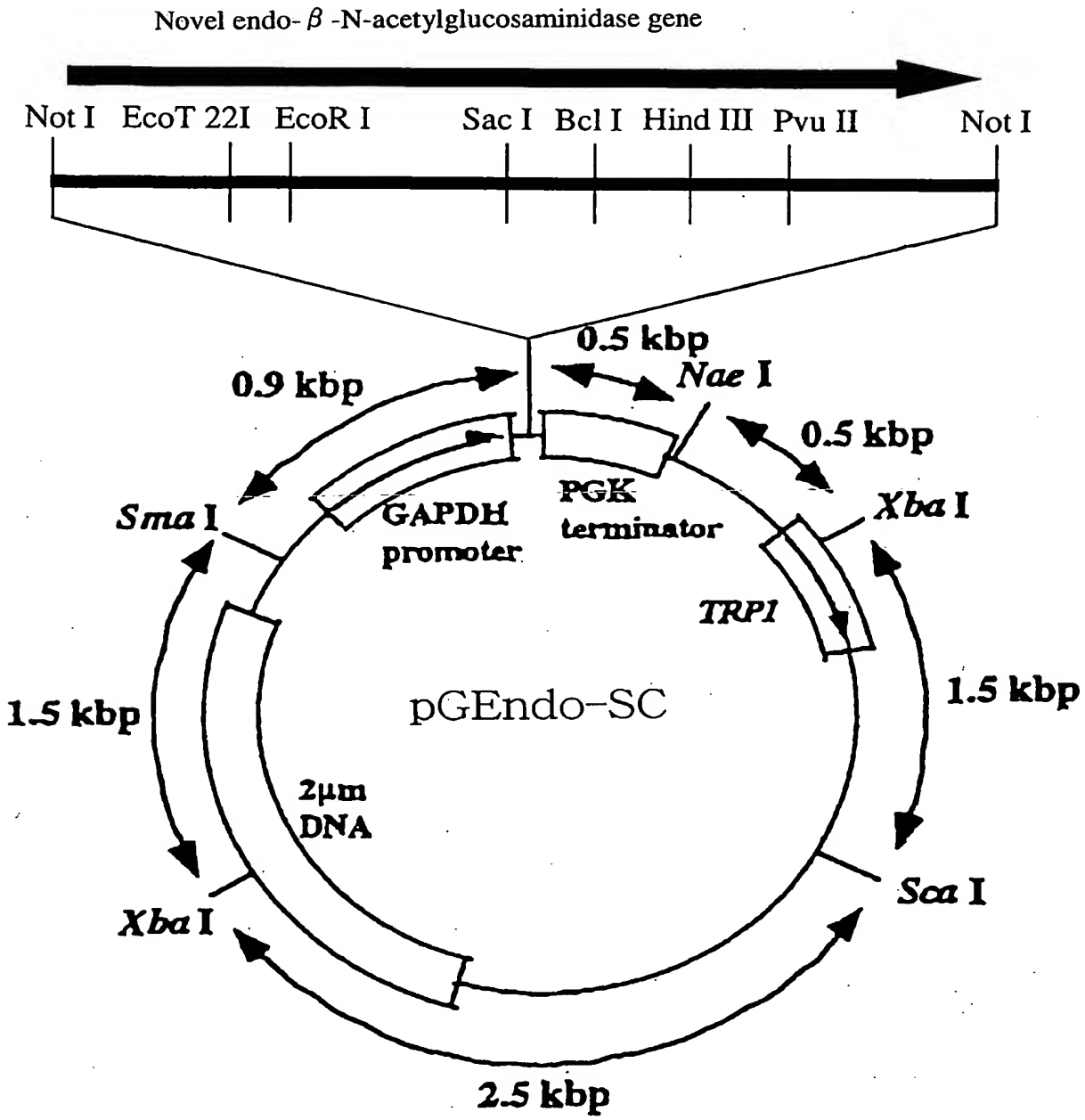
Amino acid sequence deduced from the novel Endo-β-N-acetylglucosaminidase gene, and the nucleotide sequence of the DNA encoding this amino acid sequence. (Continued)

FIG. 7

```
        1467        1476        1485        1494        1503        1512
AAA GGA TGC TCA TTG CGT TAT ATT TAT AGA ACT TTG TTG ATG AAA GAT GTA AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   G   C   S   L   R   Y   I   Y   R   T   L   L   M   K   D   V   K

        1521        1530        1539        1548        1557        1566
TTG ACA GTA GCA TGT CAC TTT TCG TTA AAA ACA AAC GAC TCA GTT AAT TTC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   T   V   A   C   H   F   S   L   K   T   N   D   S   V   N   F   F

        1575        1584        1593        1602        1611        1620
AAG GTA TGG CAG CCA GAT GAA AAT TTC TCT TTT GAA TAT GAT GAT GGA ATG AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   V   W   Q   P   D   E   N   F   S   F   E   Y   D   D   G   M   R

        1629        1638        1647        1656        1665        1674
GCC ACT GTT ACA ACT GAA AAT TCT ACC GAA AGC AGA TGC TTT TTA TTA CGT ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   T   V   T   T   E   N   S   T   E   S   R   C   F   L   L   R   T

        1683        1692        1701        1710        1719        1728
ACA GAA GAA GAT ACA GGA GAA AAT GAT TGG ATA ACA AAA ACT ATT AAT GTG CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   E   E   D   T   G   E   N   D   W   I   T   K   T   I   N   V   P

        1737        1746        1755        1764        1773        1782
GCT GTT CCA GAA GGA AGT CAA TTA TAC ATT ACA AGA CTT GAA GTG AGC GTA GTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   V   P   E   G   S   Q   L   Y   I   T   R   L   E   V   S   V   V

        1791        1800        1809        1818        1827        1836
TTA GAT ACA GCT GGT TTA GTA GGT CTT GTT AAT CAA GTT ATT GCT TGC TTG GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   D   T   A   G   L   V   G   L   V   N   Q   V   I   A   C   L   G

        1845        1854        1863        1872        1881        1890
TAT ATT AGC ATC ATA CCA ACT ATA AAT TCT GGA ATA AAA ACA GAT TCA TCA CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   I   S   I   I   P   T   I   N   S   G   I   K   T   D   S   S   R

        1899        1908        1917        1926        1935        1944
ATT ATT CAG GAT CTC TTT TGG AAA GAT CAG AAA TAT ACC AAA ATC GGA AAA GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   I   Q   D   L   F   W   K   D   Q   K   Y   T   K   I   G   K   E

        1953        1962        1971        1980        1989        1998
AGT TTA GAC GAC ATA GCT CAA GAA GAA GTT CAT AGA TAT TAT GGA ACA TTG AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   L   D   D   I   A   Q   E   E   V   H   R   Y   Y   G   T   L   N

        2007        2016        2025        2034        2043        2052
TGG GAA AAC ACA GCA AAT GTA GTA AAC GCT TGG GAG GAA ATA GAT TAC TAC AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 W   E   N   T   A   N   V   V   N   A   W   E   E   I   D   Y   Y   N

        2061        2070        2079        2088        2097        2106
GTT TTT TAC AAA GAA AGT GAC GAC TCT GCA ACT CGC ATC TTT TTA GGA ACA GCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   F   Y   K   E   S   D   D   S   A   T   R   I   F   L   G   T   A

        2115        2124        2133        2142        2151        2160
TTC TGT AAT CAA TTT CGT GTA TCT GGT TTA GAT ATT ATT TTA TCT AAG CTA CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   C   N   Q   F   R   V   S   G   L   D   I   I   L   S   K   L   P

        2169        2178        2187        2196        2205        2214
AAG ATA GTT ATT GAA GCT GTT AAC AAA GAA GGA TAC ATC TCT TCA AGT GGT AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   I   V   I   E   A   V   N   K   E   G   Y   I   S   S   S   G   S

        2223        2232
ATA GAT TTG TCA TTA AAC TAG 3'
--- --- --- --- --- --- ---
 I   D   L   S   L   N   *
```

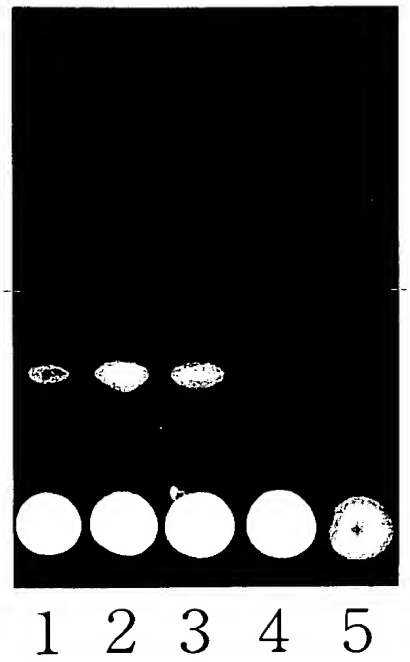
Amino acid sequence deduced from the novel Endo-β-N-acetylglucosaminidase gene, and the nucleotide sequence of the DNA encoding this amino acid sequence. (Continued)

FIG. 8



Structure of expression vector pGEndo-SC for the use in *Saccharomyces cerevisiae*, which comprises a novel endo-β -N-acetylglucosaminidase gene.

FIG. 9



Expression of endo-β -N-acetylglucosaminidase enzyme in yeast into which an endo-β -N-acetylglucosaminidase gene has been introduced.

Lanes 1-3: Cellular extract of *S. cerevisiae* YPH500 (pep4) into which an endo-β -N-acetylglucosaminidase gene has been introduced.

Lane 4: Purified endo-β -N-acetylglucosaminidase derived from *M. hiemalis*

Lane 5: Cellular extract of *S. cerevisiae* YPH500 (pep4)